SLOVENSKI STANDARD
oSIST prEN ISO 14501:2020

01-februar-2020

Mleko in mleko v prahu - Določevanje vsebnosti aflatoksina M1 - Čiščenje z imunoafinitetno kromatografijo in določevanje s tekočinsko kromatografijo visoke ločljivosti (ISO/DIS 14501:2019)

Milk and milk powder - Determination of aflatoxin M1 content - Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography (ISO/DIS 14501:2019)

Milch und Milchpulver - Bestimmung des Gehalts an Aflatoxin M1 - Reinigung durch Immunaffinitäts-Chromatographie und Bestimmung mit Hochleistungs-Flüssigchromatographie (ISO/DIS 14501:2019)

Lait et lait en poudre - Détermination de la teneur en aflatoxine M1 - Purification par chromatographie d'immunoaffinité et détermination par chromatographie en phase liquide à haute performance (ISO/DIS 14501:2019)

Ta slovenski standard je istoveten z: **prEN ISO 14501**

ICS:

67.100.10	Mleko in predelani mlečni proizvodi	Milk and processed milk products

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Milk and milk powder — Determination of aflatoxin M1 content — Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography

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Forewords

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products* and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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This document was prepared by the IDF *Standing Committee on Analytical Methods for Composition* and the ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

The work was carried out by the IDF-ISO Action Team on A12 of the *Standing Committee on Analytical Methods for Composition* under the aegis of its project leader Mr. Paul Jamieson.

Milk and milk powder — Determination of aflatoxin M1 content — Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography

1 Scope

This document specifies a method for the determination of aflatoxin M_1 content in milk and milk powder. The lowest level of validation is 0,08 µg/kg for whole milk powder i.e. 0,008 µg/l for reconstituted liquid milk. The limit of detection (LOD) is 0,05 µg/kg for milk powder and LOD is 0,005 µg/kg for liquid milk. The limit of quantification(LOQ) is 0,1 µg/kg for milk powder and LOQ is 0,01 µg/kg for liquid milk.

The method is also applicable to low fat milk, skimmed milk, low fat milk powder and skimmed milk powder.

CAUTION 1 The method described in this protocol requires the use of solutions of aflatoxin M_1. Aflatoxins are carcinogenic to humans. Attention is drawn to the statement made by the International Agency for Research on Cancer (WHO)[1,2].

CAUTION 2 Protect the laboratory in which the analyses are performed adequately from daylight and keep aflatoxin standard solutions protected from light, e.g. by using aluminium foil.

CAUTION 3 The use of non-acid-washed glassware (e.g. tubes, vials, flasks, beakers, syringes) for aqueous aflatoxin solutions may cause loss of aflatoxin.
Moreover, brand new laboratory glassware, before coming into contact with aqueous solutions of aflatoxin, should be soaked in dilute acid (e.g. sulfuric acid, 2 mol/l) for several hours, then rinsed well with distilled water to remove all traces of acid (check to ensure pH is in the range 6 to 8).

CAUTION 4 Use decontamination procedures for laboratory wastes such as solid compounds, solutions in organic solvents, aequeous solutions and spills, and for glassware coming into contact with carcinogenic materials. Suitable decontamination procedures have been developed and validated by the International Agency for Research on Cancer (WHO)[1,2].

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
Aflatoxin M_1 content
Concentration (in µg/l) or mass fraction (in µg/kg) of substances determined by the procedure specified in this document.

4 Principle

Aflatoxin M_1 is extracted by passing the test portion through an immunoaffinity column that contains specific antibodies bound onto a solid support material.

As the sample passes through the column, the antibodies are selectively bound with any aflatoxin M_1 (antigen) present and form an antibody-antigen complex. All other components of the sample matrix are washed off the column with water. Then aflatoxin M_1 is eluted from the column and the eluate is collected. The amount of aflatoxin M_1 present in this eluate is determined by means of high-performance liquid chromatography (HPLC) coupled with fluorimetric detection.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

5.1 Immunoaffinity column

The immunoaffinity column shall contain antibodies against aflatoxin M_1. The column shall have a maximum capacity of not less than 100 ng of aflatoxin M_1 (which corresponds to 2 µg/l when a volume of 50 ml of a test portion is applied). It shall give a recovery of not less than 80 % for aflatoxin M_1 when a standard solution containing 4 ng of toxin is applied (which corresponds to 80 ng/l when a volume of 50 ml of sample is applied). Any immunoaffinity column meeting the performance specifications mentioned above can be used.[1)]

The performance of the columns shall be checked regularly and at least once for every batch of columns (see the procedure in 5.1.1 and 5.1.2).

5.1.1 Capacity check

Dilute 2,0 ml of aflatoxin M_1 standard stock solution (5.4.2) to 50 ml with water. Mix well and apply the whole volume to the immunoaffinity column carefully following the recommendations given by the manufacturer for the use of columns. Wash the column and elute the toxin. Determine the amount of aflatoxin M_1 eluted from the column by HPLC after preparing a suitable dilution of the final eluate.

Calculate the capacity for the aflatoxin. Compare the result with the requirements given in 5.1.

5.1.2 Recovery check

Use a pipette (6.4) to dilute 0,8 ml of aflatoxin M_1 standard working solution of 0,005 µg/ml (5.4.3) to 50 ml with water. Mix well and apply the whole volume to the immunoaffinity column carefully following the recommendations given by the manufacturer for the use of columns. Wash the column and elute the toxin. Determine the amount of aflatoxin M_1 eluted from the column by HPLC after preparing a suitable dilution of the final eluate.

Calculate the recovery for the aflatoxin. Compare the result with the requirements given in 5.1. The concentration shall not be less than 0,064 µg/l. Recovery checks can also be conducted with commercially available reference materials.

1) Examples of immuno affinity columns: Afla Test P (Vicam®), Aflaprep® M (R-Biopharm), similar products are also available from Romer Labs®, Bioo Scientific® and Neogen®, these products are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO and/or IDF of these products.